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COMMITTEE ON FOOD RESEARCH**

U.S. QUARTERMASTER FOOD AND CONTAINER INSTITUTE
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**RESEARCH AND DEVELOPMENT BRANCH
MILITARY PLANNING DIVISION
OFFICE OF THE
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SUMMARY

1. Most of the rats were able to maintain their weight or gain slightly in a self-selection maintenance situation.

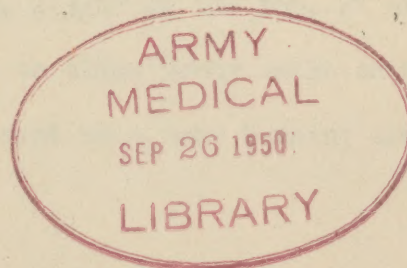
2. The selections of most nutrients varied greatly from day to day and week to week within the same cage and also between the two groups of litter-mate rats. Of special interest is the fact that in one cage average casein intake remained quite constant; in the other cage it fell off gradually but persistently.

3. Because of the marked variations it is concluded that self-selection maintenance is not a technique that lends itself to discerning small but significant changes in intakes unless very large numbers of rats are used. This would vary, however, depending on the nutrient, for the intakes of some substances were much more stable than others.

4. "Positional eating" may occur with group feeding, contrary to the observation of Young (J. Comp. Psych. 37 371, 1944). While it is not a constant finding its occurrence makes random changes in the positions of the solid foods necessary.

5. The amount of intake of nutrients is apparently regulated largely by the volume of the solution that the rat chooses to take and not by the concentration. Changes in concentration do not reflect themselves in similar changes in volume. With the possible exception of riboflavin the vitamin intake was far above physiological requirements.

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(Continued)

SELF-SELECTION EXPERIMENT

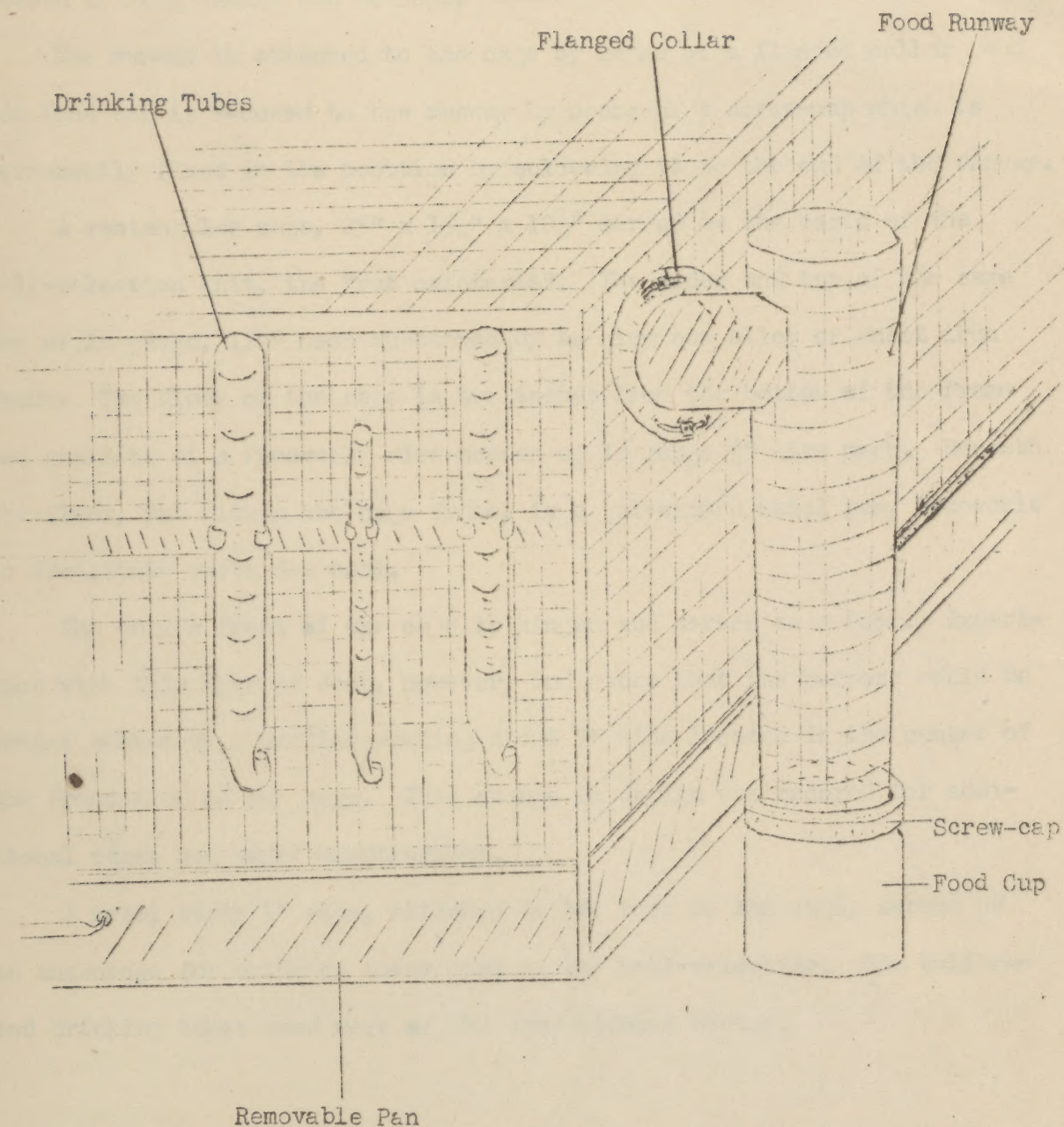
I. Description of Cages.

The most serious problem in cage design for self-selection experiments is that of providing for accurate measurement of solid food intake. Rats have a habit of carrying food in their paws and in their mouths from the food cup to some part of the cage where it drops through the floor screening into the shavings, making accurate measurement of food intake impossible.

A modification of an idea suggested to us by Dr. E. C. Albritton of the Department of Physiology of George Washington University, Washington, D. C., was tried and proved satisfactory. In his self-selection cages Dr. Albritton employs truncated, cone-shaped, inclined runways which are located inside the cage and at the bottom of which are food cups. This device forces the rat to back up when leaving the cup, and in so doing its front paws must be used for balance and motion, depriving the animal of the opportunity of carrying food.

In our cages (Figure 1), the runway is placed outside the cage for two reasons: (1) to reduce the food loss to a still greater extent, and (2) to eliminate the necessity of opening the cage whenever food cups have to be replaced. The runway was built of wire mesh in the shape of a truncated cone. The measurements, $2\frac{3}{4}$ " at the top, 2" at the bottom over a length of 7", were chosen so as to allow young adult animals to crawl through the runway and yet to prevent them from turning around once there.

FIGURE 1: Representative Portion of Self-Selection Unit Showing Essential Elements.



On our cages the runways are placed in a vertical position with the wider diameter at the top, forcing the rats to use their front legs for balance and motion and so preventing them from carrying food back into the cage. Food scattered or dropped is collected in a container surrounding the food runway to half its length. With this device an accurate record of food intake can be kept.

The runway is attached to the cage by means of a flanged collar and the food cup is secured to the runway by means of a screw-cap which is permanently fixed in its position by soldering it to the end of the runway.

A rectangular cage, 25" x 15 $\frac{1}{2}$ " x 10 $\frac{1}{2}$ " serves as the basis of the self-selection unit, the "rat cafeteria". The sides and top of the cage are of 18 gauge, 1/4" mesh sustained by an aluminum alloy or sheet iron frame. The floor of the cage is two inches from the bottom of the frame and consists of a removable wire screen of 16 gauge $\frac{1}{2}$ " wire mesh. Beneath the floor, yet within the cage frame, is a galvanized metal pan, removable to facilitate waste disposal.

The entire front of the cage is hinged and serves as a door. Experience with this type of door, however, indicates that its purpose would be better served by a smaller opening about 7" wide located in the center of the front side of the cage. This change in design was ordered for additional cages now under construction.

A metal strip 1" wide, attached to the rear of the cage, serves as an anchorage for drinking tubes used in the self-selection. The calibrated drinking tubes used were of the conventional design.

II. Weight maintenance.

The ability of the rats to maintain their health and weight in the "rat cafeteria" was investigated first.

Twelve 120 day old female rats of the Sherman strain were used. These were distributed between two cages, three each of two litters in each cage.

The purified nutrients offered were as follows:

CaCl ₂ - 2% solution	Thiamine - (1) 200 mcg/ml (2) 50 mcg/ml
KCl - 1% solution	Riboflavin - (1) 25 mcg/ml (2) 50 mcg/ml
MgSO - 0.5% solution	Niacin - (1) 1000 mcg/ml (2) 100 mcg/ml
Tap Water	Pyridoxine - (1) 200 mcg/ml (2) 100 mcg/ml
Na ₂ HPO ₄ - (1) 4% (2) 6%	Ca Pantothenate - (1) 100 mcg/ml
NaCl - 3%	Choline chloride - 3000 mcg/ml
Biotin - (1) 5 mcg/ml (2) 0.1 mcg/ml	Corn oil
Vitamin A (in corn oil) 100 I.U./ml	
Casein, vitamin free - as a solid	
Cerelose - as a solid	

The nutrients in liquid form were offered in calibrated tubes of capacities best suited to the individual concentrations used. Because of the limited capacity of the food cups, two food runways were set up for each solid nutrient.

Weights have been recorded at weekly intervals for a five week period and are tabulated in Table I.

Only eight of the twelve rats gained weight in this period, two maintained their weight and two lost weight. The rats are being continued on self-selection.

TABLE 1: Weights of Rats Before and During the Five Week Self-Selection Period.

Cage I	Rat #	Before Self-Selection	1st Week	2nd Week	3rd Week	4th Week	5th Week
		Gms.	Gms.	Gms.	Gms.	Gms.	Gms.
Litter 1	1	197	205	211	206	205	197
"	2	189	198	207	210	204	207
"	3	200	209	219	224	221	215
Litter 2	4	193	204	214	216	213	213
"	5	177	187	188	183	176	170
"	6	155	165	176	177	176	183
Cage II							
Litter 2	7	182	178	190	182	181	179
"	8	202	212	220	224	229	223
"	9	171	174	185	182	185	179
Litter 1	10	207	217	219	210	200	198
"	11	211	230	240	237	240	243
"	12	195	203	208	200	202	216

III Intake Variation:

In the initial five week period during which the rats were on self-selection maintenance, the daily intakes of each of the 17 nutrients offered (listed on page 4) were recorded for each of the two groups.

The extent of the variations of each of the nutrients within each cage and between cages is presented in three different ways:

(1) Expressing the range of intake of each nutrient in each cage in terms of percentage variation from the minimum intake (Table 2).

(2) Charting the average daily intakes of the nutrients on a weekly basis (Fig. 2).

(3) Calculation of the standard deviations for each nutrient for each week (Table 3).

Examination of these data shows marked variation of intake for most of the nutrients. Thus in terms of average percentage variation from the minimum intake (Table 2) only Na_2HPO_4 , NaCl and cerelose have a range below 100%, while KCl, vitamin A in corn oil, riboflavin, pyridoxine, corn oil and casein have ranges between 100% and 200%. The ranges of the remaining nutrients varied up to 810% of the minimum intake, as in the case of biotin.

TABLE 2: Range of Weekly Mean Intakes of Nutrients; expressed as percentage variation from the minimum intake.

Nutrient	Group I	Group II	Average	Nutrient	Group I	Group II	Average
	%	%	%		%	%	%
CaCl_2	600	40	320	Riboflavin**	160	110	135
KCl	110	225	168	Niacin**	475	50	260
MgSO_4	180	620	400	Pyridoxine**	250	85	165
H_2O	310	200	255	Ca Pan	350	325	335
$\text{Na}_2\text{HPO}_4^*$	60	90	75	Choline	280	500	390
NaCl	90	80	85	Corn Oil	80	200	140
Biotin**	1500	120	310	Cerelose	14	25	19.5
Vit. A (in corn oil)	85	125	105	Casein	200	25	110
Thiamine**	770	300	535				

* Over a 4 week period at one concentration

** Over a 3 week period at one concentration

A
Niacin
Ca Pantothenate
Pyridoxine

B
Tap water
Casein
CaCl₂

C
Na₂HPO₄
Biotin

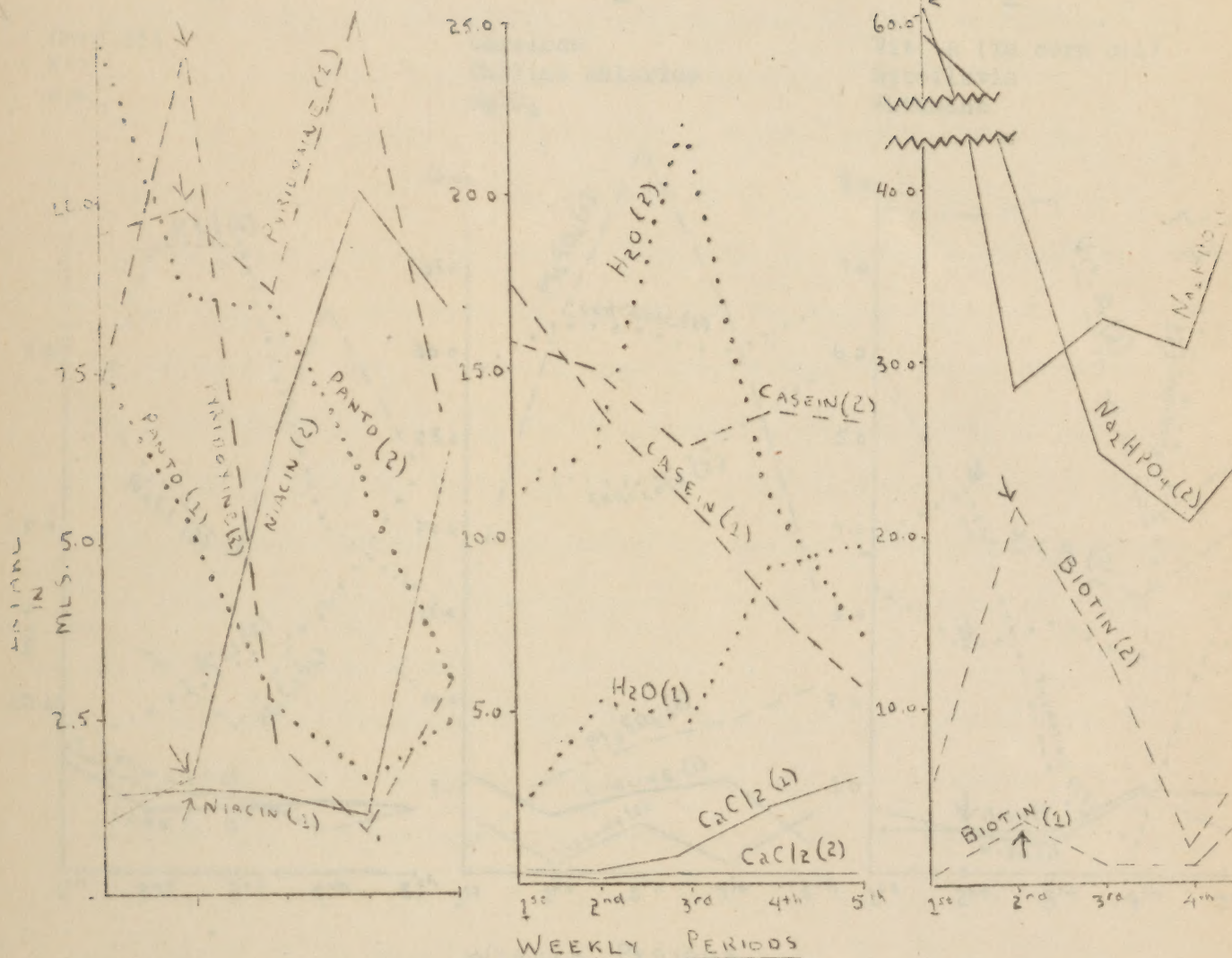


FIGURE 2: Average Daily Intake for 6 Rats for Each of 5 Weekly Periods: subscripts following each nutrient refer to cage number. Arrows refer to time of changes in concentration (see text) as follows:

Riboflavin	-	from 25 mcg/ml to 50 mcg/ml.
Thiamine	-	" 200 " " " 50 " "
Niacin	-	" 1000 " " " 100 " "
Pyridoxine	-	" 200 " " " 100 " "
Biotin	-	" 5 " " " 0.1 " "
Na ₂ HPO ₄	-	" 4% to 6%

D

Corn oil
KCl
NaCl

E

Cerelose
Choline chloride
MgSO₄

F

Vit. A (in corn oil)
Riboflavin
Thiamine

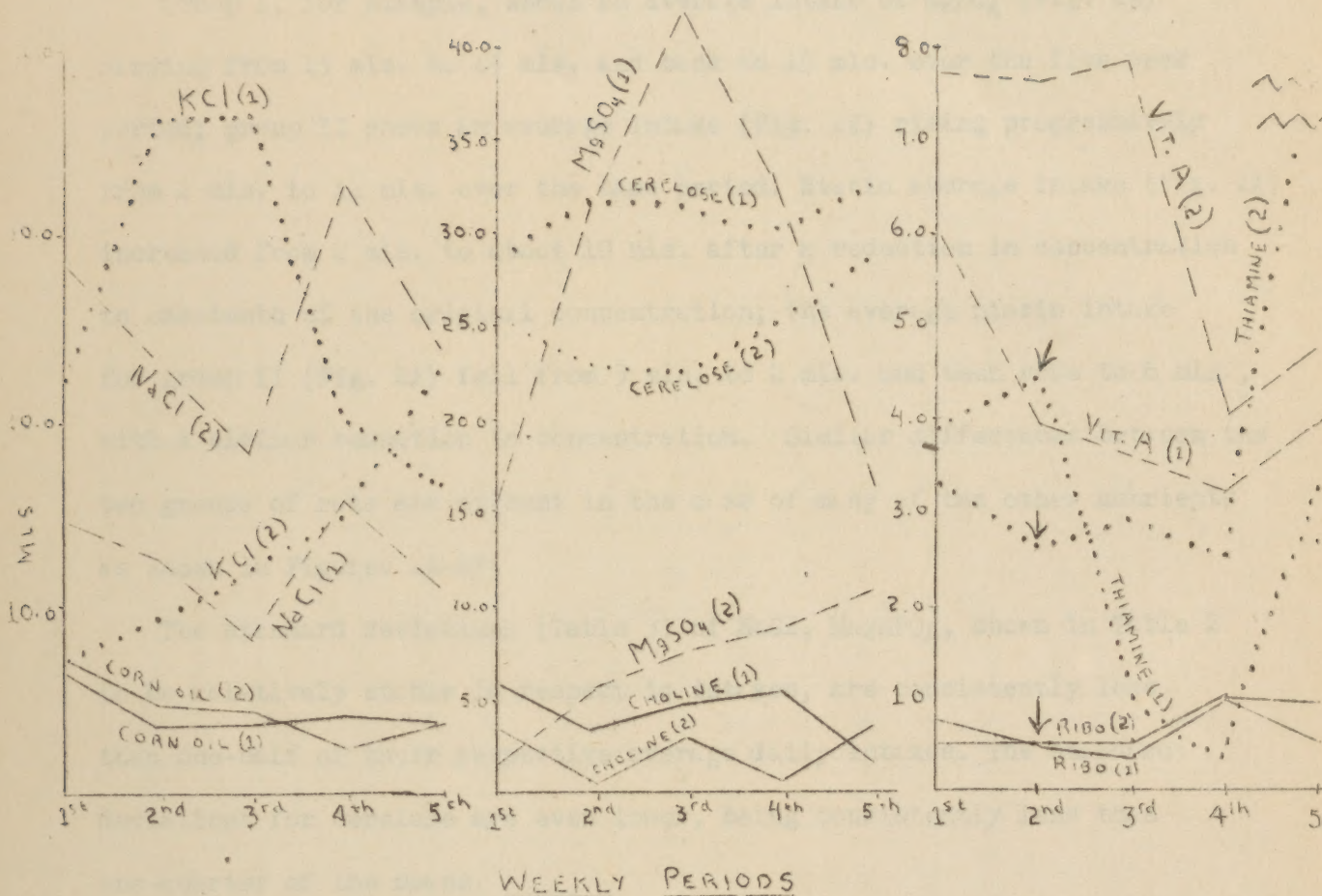


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Riboflavin	-	from 25 mcg/ml to 50 mcg/ml
Thiamine	-	" 200 " " " 50 " "
Niacin	-	" 1000 " " " 100 " "
Pyridoxine	-	" 200 " " " 100 " "
Biotin	-	" 5.0 " " " 0.1 " "
MgHPO ₄	-	" 4% to 6%

The differences between the two groups of rats in the intake of particular nutrients are striking; in many instances these differences are greater than the variations between nutrients.

Group I, for example, shows an average intake of MgSO_4 (Fig. 2E) varying from 15 mls. to 45 mls. and back to 15 mls. over the five week period; group II shows an average intake (Fig. 2E) rising progressively from 2 mls. to 12 mls. over the same period. Niacin average intake (Fig. 2A) increased from 2 mls. to about 10 mls. after a reduction in concentration to one-tenth of the original concentration; the average niacin intake for group II (Fig. 2A) fell from 3 mls. to 2 mls. and then rose to 6 mls., with a similar reduction in concentration. Similar differences between the two groups of rats are evident in the case of many of the other nutrients as shown in Figures 2A-2F.

The standard deviations (Table 3) of NaCl , Na_2HPO_4 , shown in Table 2 to be relatively stable in respect to intakes, are consistently less than one-half of their respective average daily intakes. The standard deviations for cerelese are even lower, being consistently less than one-quarter of the means.

Standard deviations for these three nutrients seem to show little variability. Standard deviations for the other nutrients varied considerably. For example, the standard deviations of biotin vary from 50% of the mean to 20% greater than the mean in group I; in group II from 50% of the mean to 100% greater than the mean. Intake of thiamine shows standard deviations which vary from 30% of the mean to 500% greater than the mean in group II; from 75% of the mean to 25% greater than the

TABLE 3: Standard Deviations From Mean Daily Intakes Per Cage For Each of 5 weeks. Odds.

* Changes made in concentration from that of preceding weeks (see fig. 2).

Cage #	1st week						2nd week						3rd week						4th week						5th week					
	m	sd	m	sd	m	sd	m	sd	m	sd	m	sd	m	sd	m	sd	m	sd	m	sd	m	sd	m	sd	m	sd	m	sd	m	sd
Nutrients																														
CeCl ₂	.46	.14	.31	.03	.44	.09	.4	.01	.89	.77	.75																			
KCl	10.6	10.3	6.8	3.4	36.0	3.3	9.2	7.0	36.3	6.4	12.0	2.2	20.6	8.7	14.7	6.6	16.3	2.3	21.3	4.2	2.0	.31	.27	5.1	5.3	.42	.28			
MgSO ₄	15.2	6.5	1.5	.88	21.4	10.7	5.5	4.5	42.7	11.0	7.3	6.2	32.1	15.9	8.7	6.4	16.4	10.6	10.9	5.6										
Tap Water	2.3	1.7	11.4	6.5	5.2	4.1	12.7	5.6	4.9	2.7	21.9	3.4	9.1	7.0	11.1	5.6	9.6	4.6	7.1	3.2										
Na ₂ HPO ₄	60.6	13.5	58.4	9.7	23.0	3.7	40.3	21.3	32.1	7.3	24.2	7.1	20.3	3.3	20.9	5.9	44.4	10.9	28.1	9.2										
NaCl	14.5	3.3	28.1	11.2	12.4	5.3	23.5	8.1	8.2	1.7	18.2	4.7	15.8	6.0	32.6	9.8	11.0	5.4	23.9	6.6										
Biotin	.2	.1	5.7	4.3	3.2	3.8	21.1	8.6	.97	.95	13.0	7.7	*.98	1.0	1.5	2.9	5.9	5.0	9.0	4.3										
Vit A (in corn oil)	5.9	2.1	7.7	1.4	3.9	.18	7.6	.28	3.6	.53	7.8	2.7	3.2	.6	4.0	2.9	3.9	1.3	4.7	.85										
Thiamine	3.9	2.2	3.3	3.8	4.4	4.9	4.6	14.2	*.99	.62	2.2	2.1	.36	.25	2.5	5.3	3.2	2.5	10.1	3.6										
Riboflavin	.71	.5	.46	.46	.41	.22	.44	.77	*.34	.43	.43	.14	.9	.37	.89	.63	.43	.1	.9	.45										
Niacin	1.3	1.1	.9	.51	1.5	1.8	2.1	1.5	*.1	1.3	6.5	4.6	1.1	2.2	10.2	3.6	6.4	7.0	8.1	6.5										
Pyridoxine	7.2	4.5	2.5	4.1	12.0	6.5	9.9	2.5	*.2	3.5	8.6	12.2	.93	.8	12.7	4.6	3.1	1.8	6.1	6.1										
Ca Pan	7.8	2.9	12.5	3.8	5.4	1.9	8.7	6.3	2.7	2.8	3.5	2.2	1.7	1.4	6.0	7.0	2.5	1.4	2.1	2.2										
Choline	5.7	8.0	3.6	2.4	3.3	2.9	.66	1.2	4.9	2.3	3.0	3.1	5.1	5.1	.8	2.8	1.5	1.1	3.1	2.0										
Corn oil	6.3	2.1	7.2	2.9	3.5	.85	4.9	1.7	3.7	1.2	4.3	.71	4.0	1.2	2.4	1.5	3.8	1.5	3.1	1.7										
Cerelose	18.6	2.8	15.1	5.9	21.9	7.8	22.3	6.0	21.3	4.5	22.6	3.3	20.1	2.9	25.0	1.2	32.7	.0	28	5.4										
Cesain	17.5	4.9	16.8	4.6	13.8	1.2	14.9	4.2	11.0	.6	12.6	2.0	8.1	1.5	12.1	1.2	5.7	.8	12	1.7										

mean in group I.

Standard deviations for KCl, vitamin A in corn oil, casein and corn oil are generally 50% of their respective means or less (Table 3). Those for CaCl_2 , MgSO_4 , water, riboflavin, niacin, calcium pantothenate, ascorbic acid and pyridoxine vary from approximately 50% of their respective means to values equal to or slightly greater than the means.

IV Positional Eating:

A study was made of the problem of "positional eating". The four food runways were numbered according to their position around the cage as follows:

- Position #1 - left front of cage
- Position #3 - left rear " "
- Position #4 - right rear " "
- Position #6 - right front " "

The solid foods, casein and cerelose, were moved as indicated below and the intake and positions were recorded daily. In cage I the positions of these foods were changed daily in a random manner for a three week period and then were kept constant for two weeks; in cage II the positions were changed daily for the entire five week period.

Table 4A shows the percentage of total weekly intake of each of these two foods at each position in cage II for the five weekly periods. It is apparent that the animals ate more of the solid foods from position 1 than from any of the others. Out of 19 possible choices, eating position 1 ranked first in intake 12 times and ranked second 6 times. This left only the one remaining choice to fit the two remaining ranks, a condition which is well above the probability due to chance sampling.

TABLE 4A: Percentage of Total Intake from Each Food Cup at Each Position.

CAGE II

Cup I-Cerelose						Cup IV-Cerelose				
Week	1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th
Positions	%	%	%	%	%	%	%	%	%	%
1	42.2	37.5	24.3	26.0	37.0	36.8	23.5	37.4	33.4	23.5
3	19.9	21.5	47.1	20.9	26.0	21.2	22.7	29.0	19.0	23.2
4	27.5	23.5	28.5	29.0	24.0	25.0	20.0	--	26.0	30.2
6	10.6	21.5	--	24.1	13.0	17.0	23.3	23.7	21.6	13.1
Cup II-Casein						Cup III-Casein				
Position	%	%	%	%	%	%	%	%	%	%
1	24.8	32.6	--	24.7	23.4	43.5	43.8	19.0	34.6	21.0
3	34.1	29.0	25.7	22.5	17.4	22.2	11.4	37.5	28.6	27.1
4	23.3	18.3	52.0	30.7	30.8	15.8	26.2	21.4	23.1	23.2
6	17.8	9.6	22.3	22.1	28.4	13.5	16.6	22.1	14.7	13.7

TABLE 4B: Percentage of Total Intake from Each Food Cup at Each Position

CAGE I

Cup I-Cerelose				Cup IV_Cerelose			Cup II-Casein			Cup III-Casein		
Week	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Positions	%	%	%	%	%	%	%	%	%	%	%	%
1	22.0	30.6	16.8	21.0	22.2	21.1	25.4	14.4	30.7	25.5	17.9	21.0
3	26.9	27.0	22.8	25.8	30.3	23.9	19.9	31.0	17.9	33.9	26.1	27.5
4	28.5	23.6	21.1	24.6	15.1	26.1	23.9	25.8	11.5	--	23.5	18.3
6	22.6	13.6	19.4	27.7	32.4	29.5	30.8	28.9	39.9	30.4	22.4	22.1

Table 4B presents a similar breakdown of the three week period in which the positions were changed in cage I. In this case the distribution of the amount of food intake is much more in accordance with random choice.

Thus, in the behaviour of the rats in one cage, there appears to be a clear-cut example of "positional eating", while in the other cage this was not so apparent.

V Changes in Concentration:

The effect of changes in concentration on intake, both liquid and solid equivalent, was next investigated. With slight variations, concentrations of the liquid nutrients first employed were those used by Richter and Hawkes (Am. J. Physiol. 131 639, 1940) and are given on page 4 under weight maintenance. Sodium phosphate (dibasic), thiamine hydrochloride, riboflavin, niacin, pyridoxine and biotin were selected for change in concentration. The results of the changes are tabulated in Table 5.

a. Effect on volume.

When the concentration of sodium phosphate was increased from 4% to 6% there was a decrease in liquid intake of 55% in cage I and a decrease in liquid intake in cage II of 48%. The concentration of thiamine was decreased to one-quarter of the original value (i.e. from 200mcg/ml to 50 mcg/ml). Following this the liquid intake decreased to one-seventh of its previous value in cage I while in cage II it decreased to one-half of its previous value . Doubling the concentration of riboflavin from 25 mcg/ml to 50 mcg/ml was followed by a slight increase in intake in cage I and a three-fold increase in cage II.

Table 5: Influence of Change in Concentration on Average Daily Intake per Animal

Conc. gts	Conc.	Cage I Intake		Cage II Intake	
		Volume	Solids	Volume	Solids
Ascorbic	4*	10.1 mls	400 mgs	9.7 mls.	39 mgs.
	63****	5.8 "	322 "	4.7 "	232 mgs.
Thiamine	200 mcgs/ml**	.69 "	139	.5	100 "
	50 " ***	.41 "	20.5 mcgs.	.68 "	43.0 "
Riboflavin	25 " **	.09 "	2.3 "	.07 "	1.9 "
	50 " ***	.06 "	3.1 "	.12 "	6.0 "
Nicotin	1000 " **	.24 "	107 "	.20 "	25 "
	100 " ***	.172 "	17.2 "	1.4 "	14.0 "
Pyridoxine	100 " **	1.6 "	323 "	1.7 "	330 "
	100 " ***	.34 "	14.0 "	1.6 "	160 "
Biotin	5 " ***	.14 "	1.1 "	.11 "	7.5 "
	0.1 " **	.57	057 "	.87 "	1.057 "

* Average over a period of one week

** Average over a period of two weeks

*** Average over a period of three weeks

**** Average over a period of ten weeks

When the concentration of niacin was reduced to one-tenth of its original value from 1000 mcg/ml to 100 mcg/ml the intake in cage I decreased 30%; in cage II, however, the reduction in concentration was followed by an increase in intake of 500%. When the concentration of pyridoxine was halved, from 200 mcg/ml to 100 mcg/ml, the intake of this vitamin was decreased by a proportionate amount in cage II while in cage I a reduction to one-tenth the original intake occurred. Reducing the concentration of biotin from 5 mcg/ml to 0.1 mcg/ml was followed by an increase in intake of 100% in cage I and a decrease in intake of 150% in cage II.

b. Effect on intake of nutrient.

As the concentrations of thiamine, niacin, pyridoxine and biotin were decreased, the intakes of the nutrients decreased in all instances, but the percentage of decrease varied greatly and the pattern of intake in the two cages was entirely different. As the concentration of riboflavin was increased the nutrient intake increased slightly in one cage and markedly in the other. However, as the concentration of Na_2HPO_4 was increased, nutrient intake decreased in both cages.

VI Conclusions:

1. Most of the rats were able to maintain their weight or gain slightly in a self-selection maintenance situation.

2. The selections of most nutrients varied greatly from day to day and week to week within the same cage and also between the two groups of litter-mate rats. Of special interest is the fact that in one cage average casein intake remained quite constant; in the other cage it fell off gradually but persistently.

3. Because of the marked variations it is concluded that self-selection maintenance is not a technique that lends itself to discerning small but significant changes in intakes unless very large numbers of rats are used. This would vary, however, depending on the nutrient, for the intakes of some substances were much more stable than others.

4. "Positional eating" may occur with group feeding, contrary to the observation of Young. * . While it is not a constant finding its occurrence makes random changes in the positions of the solid foods necessary.

5. The amount of intake of nutrients is apparently regulated largely by the volume of the solution that the rat chooses to take and not by the concentration. Changes in concentration do not reflect themselves in similar changes in volume. With the possible exception of riboflavin the vitamin intake was far above physiological requirements.

* Young, P.T. - J. Comp. Psych. 37 371, 1944

TASTE THRESHOLD EXPERIMENT

Richter (Harvey Lectures 38 63, 1942-3) studied the intake by rats of solutions of various toxic substances offered in gradually increasing concentrations in a simple choice situation with water as the alternative. It was thought worthwhile to repeat and extend some of his experiments.

Mercuric chloride was the first substance studied. Three male rats approximately 120 days old, of the Sherman strain, were used, each in a separate cage.

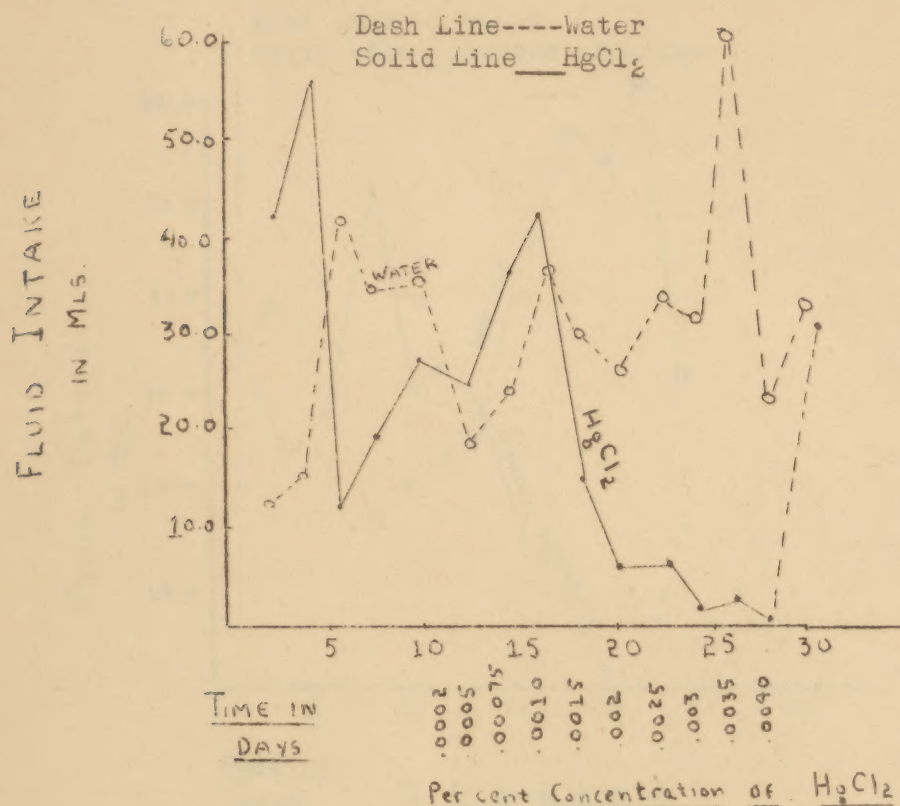
Before offering the mercuric chloride an attempt was made to equalize the water intake from each of the two tubes in order to rule out the effects of "positional" selection or other idiosyncracies. Tube positions were changed in a random manner. At the end of eight days total intakes were as follows:

	Tube #1	Tube #2
Rat #1	204 mls.	216 mls.
Rat #2	238 "	177 "
Rat #3	332 "	220 "

Except in the case of Rat #1, equalization did not take place. Mercuric chloride was therefore introduced into that tube showing the greatest intake so that any decrease would be more readily detected.

In the next 20 days each of the three rats were given mercuric chloride solutions of regularly increasing concentrations. Each concentration of mercuric chloride was offered for a two-day period followed by water as a check after every two succeeding concentration levels. Tubes were changed in a random manner.

FIGURE 3: Effect of Increasing Concentration of HgCl_2 on Intake



The concentration of the solution was increased from 0.0002% to 0.004% when intake of mercuric chloride stopped. At this point water was substituted for the mercuric chloride.

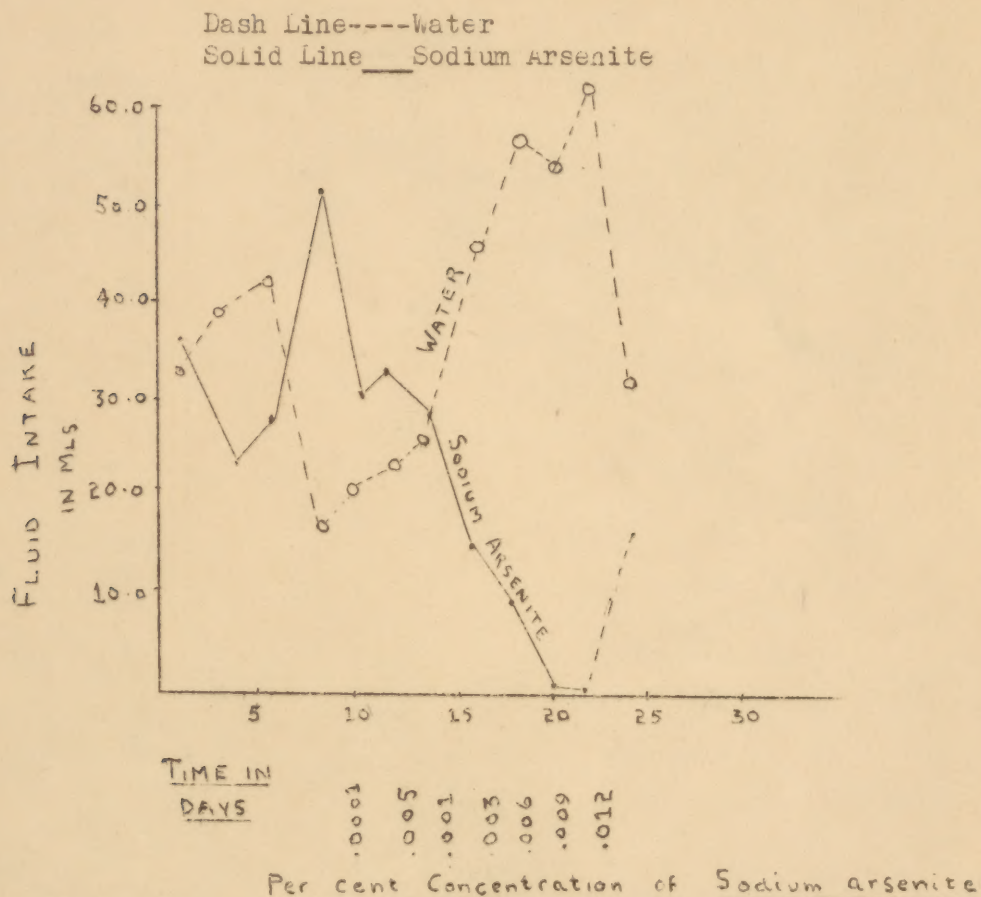
Figure 3 shows the intakes of mercuric chloride solutions and of water as the concentration of the former was increased.

It can be seen that the intake of mercuric chloride decreased sharply as the concentrations increased beyond 0.001%. At this point there is an immediate increase in water intake showing the response to a substitution of water for mercuric chloride.

These results closely follow those reported by Richter.

In a similar manner the taste threshold for sodium arsenite was investigated (Figure 4). As the concentration of sodium arsenite was increased from 0.0001% to 0.012%, intake decreased from 30 mls.

FIGURE 4: Effect of Increasing Concentration of Sodium Arsenite on Intake



to about zero. Water intake over the same period increased from 20 mls. to 60 mls.

When water was substituted for the sodium arsenite, on the 22nd day, intake from this tube increased to 15 mls. The intake from the original water tube decreased proportionately at the same time.

In general the pattern for sodium arsenite follows that of mercuric chloride quite closely.